STUDIES ON *MENTHA* × *DUMETORUM* SCHULT. (LABIATAE) TO CONFIRM ITS PRESUMED PARENTAGE — *M. AQUATICA* LINN. × *M. LONGIFOLIA* (LINN.) HUDS.

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ABSTRACT

A study of synthesized hybrids and wild strains of the species of *Mentha* has confirmed the origin and parentage of *M.* × *dumetorum* Schultes, (*M. aquatica* × *M. longifolia*). The study was based on morphology, chromosome number, meiotic behaviour, and pollen and seed fertility.

Chromosome numbers of $2n = 60$, 72, were obtained for *M.* × *dumetorum*. The interspecific hybrids showed 100% sterility correlated with meiotic irregularities.

Mentha is one of the larger and taxonomically more complex genera of the Labiatae. Briquet (1897) recognised only 15 species in the genus. Most of these species are involved in the formation of hybrids, which accounts in large measure for the extensive variation and taxonomic complexities in the genus.

Though there is considerable literature on hybrids in the genus *Mentha,* that with a bearing on the origin of *Mentha* × *dumetorum* is sparse. The object of this paper was to compare several wild strains of this hybrid with synthesized strains of known parentage using morphology, chromosome number, meiotic behaviour, and pollen and seed fertility in order to understand and confirm the origin of *M.* × *dumetorum*.

Mentha × *dumetorum* is believed to be a hybrid between *M. aquatica* L . ($2n = 96$) and *M. longifolia* L. ($2n = 24$ and 48) and is intermediate in morphology and chromosome number between the two species. These species have also been studied for comparative purposes. Schultes (1809) was the first to describe this hybrid and considered it to be intermediate between *M.* × *nemorosa* Willd. (i.e. *M.* × *villosa* Huds.) and *M. sylvestris* L. (i.e. *M. longifolia*). A fuller description of this hybrid is also given in Clapham, Tutin, and Warburg (1960).

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MATERIALS AND METHODS

The plant materials used were collected in the wild in Britain and North America by Professor J. K. Morton, and also obtained from various botanical gardens. The artificially produced hybrid strains were synthesized by Dr. M. J. Murray (A. M. Todd Company, Kalamazoo, Michigan, U.S.A.), who crossed a male-sterile strain of *M. aquatica* with fertile diploid and tetraploid strains of *M. longifolia*. Table I gives the list of the plant materials investigated.

The plants were brought into cultivation by transplanting the rhizomes or by growing from seeds. During winter they were grown in pots in the green houses to protect them from the severity of the climate at Waterloo, Ontario, Canada. In summer they were grown outside in the experimental garden under relatively uniform conditions.

Chromosome counts were made from root-tip preparations using standard acetocarmine technique. Young flower buds were used to study meiotic behaviour and the development of the pollen. The flower buds were fixed for 12 to 36 hours in either Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform, and 1 part glacial acetic acid), or in 1:3 acetic-alcohol to which ferric acetate had been added to intensify staining. They were then transferred to 70% alcohol, in which they could be stored for several days. The anthers were subsequently squashed in 2% acetocarmine. Slides were made permanent using McClintock's method (in Johansen, 1940). "Euparal" was used as the mountant.

Pollen fertility was determined by staining the pollen grains with standard glycero-acetocarmine, prepared by mixing equal volumes of 50% glycerine and 2% acetocarmine.

The well stained and well formed grains were considered to be fertile, while those which were small, malformed, or shrivelled and unstained were regarded as aborted.

RESULTS

At the outset of this study we had living material of 13 strains of mints which were believed to be referable to *Mentha* × *dumetorum*. Seven were of natural origin, collected in the wild or obtained from garden sources, whilst the others were synthesized hybrids produced by Dr. Murray. In addition we had a range of herbarium material of

TABLE I. **List of Mentha Material Examined and their Chromosome Numbers**[1]

TAXON	ORIGIN	ACCESSION NO	2*n*
Species: —			
M. aquatica	Paris, B.G. (Native)	—	96
M. longifolia	Newmill, Penzance, U.K.	JKM 219	24
M. longifolia	Cambden, Maine, U.S.A. (Cultivated)	M 31	48
Hybrids: —			
M. × *dumetorum*	Leiden, B.G.	M 26 (= M 58)	72
M. × *dumetorum*	England (without precise locality)	M 72	72
M. × *dumetorum*	Dijon, B. G.	M 77	72
M. × *dumetorum*	Newmill, Penzance, U.K.	JKM 5218	72
M. × *dumetorum*	St. Just., Cornwall, U.K.	JKM 5201	72
M. × *dumetorum*	Synthesized	M 71	60
M. × *dumetorum*	Synthesized	M 73	72
M. × *dumetorum*	Synthesized	M 74	72
M. × *dumetorum*	Synthesized	M 87	60
M. × *dumetorum*	Synthesized	M 88	72
M. × *dumetorum*	Synthesized	M 90	C 84
M. × *Piperita*	Nova Scotia	NA 4264	72

[1]Voucher specimens deposited at the Biology Department, University of Waterloo, Ontario Canada.

this hybrid, its presumed parent species, and other similar hybrids. These were used in the study of the morphology.

The initial study carried out on *M.* × *dumetorum* was of its morphological characteristics. In particular it was necessary to define and identify *M.* × *dumetorum*, for it is not always readily separable from other mints, especially hairy forms of *M.* × *piperita* (*M. aquatica* × *M. spicata*).

The measurable characters used in determining materials of wild origin are the length of calyx, leaf index, inflorescence breadth, and the leaf base form. Table II gives summary of data for recorded characters in the natural and synthesized strains of *Mentha* × *dumetorum*, the pubescent forms of *M.* × *piperita*, and in the two parent species of *M.* ×*dumetorum* — *M. longifolia* and *M. aquatica*.

Leaf size, leaf shape, inflorescence structure, and other salient characters do not differ significantly between the "wild" and synthesized hybrids. The hybrids were typically intermediate with

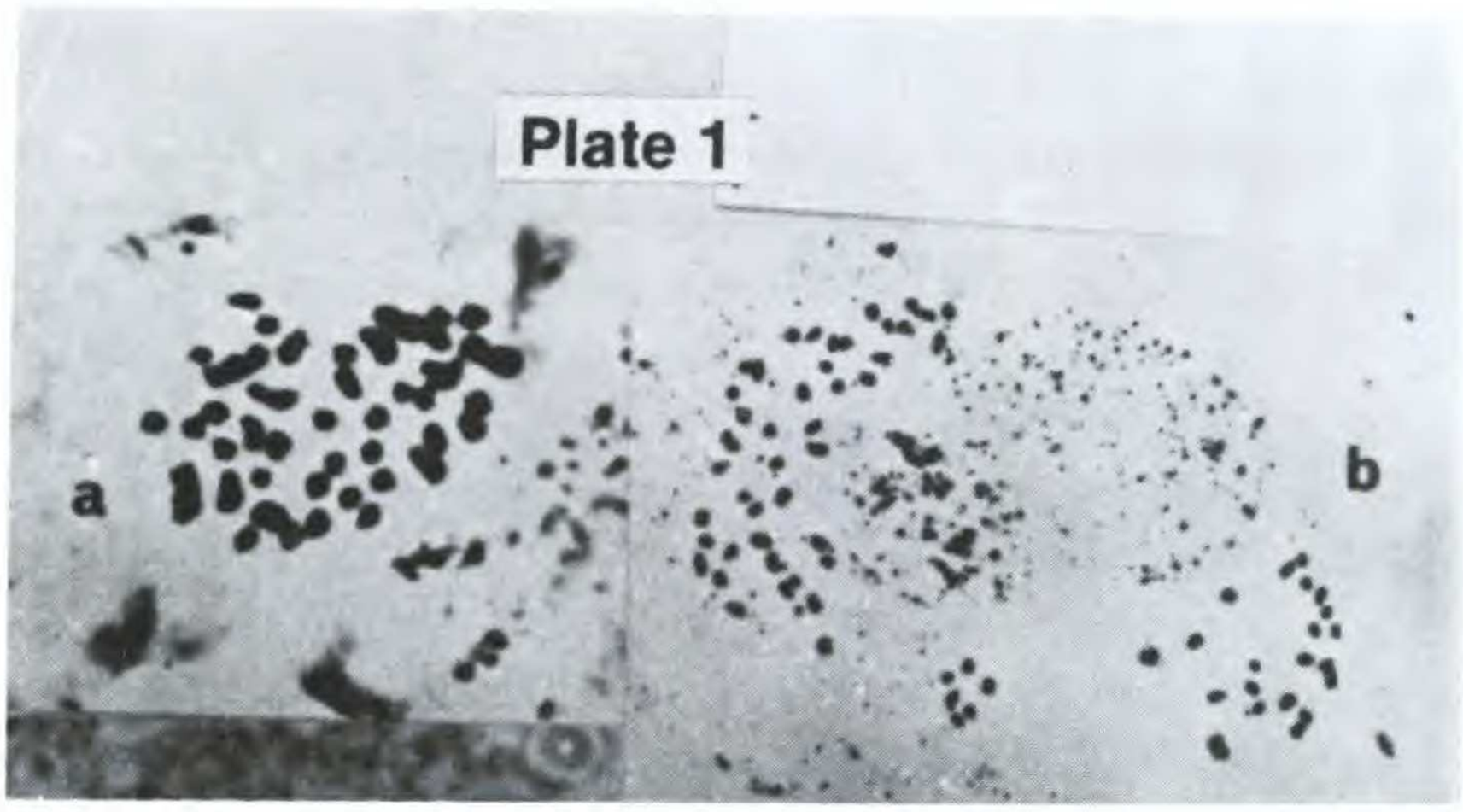
respect to the salient features of their parents, although they showed some variation in such characters as the size of leaves, and in the degree of pubescence on the leaf and stem. Thus a comparison of the artificial hybrids with those of natural occurrence showed them to be similar, and intermediate in morphological appearance between their parental species.

TABLE II. **Characters in Mentha × dumetorum, M. × Piperita and two parents of M. × dumetorum — M. longifolia and M. aquatica**

Taxon	Accession No.	2*n*	Calyx (mm)	Leaf (cm) L × B	Leaf Index L ÷ B	Inflor. Breadth (cm)	Leaf Base
M. × *dumetorum*	M26 = (58)	72	2.5	3.2 × 1.7	1.9	1.0	cuneate
M. × *dumetorum*	M72	72	2.9	4.4 × 2.3	1.9	1.4	round
M. × *dumetorum*	M77	72	2.5	5.7 × 3.2	1.8	1.6	round
M. × *dumetorum*	JKM 4583	72	2.5	5.1 × 3.0	1.8	1.4	round
M. × *dumetorum*	JKM 5201	72	2.3	4.5 × 3.0	1.6	1.4	round
M. × *dumetorum*	JKM 5202	72	2.3	4.4 × 2.8	1.6	1.4	round
M. × *dumetorum*	JKM 5218	72	2.4	5.1 × 3.4	1.6	1.4	round
M. × *dumetorum*	M71	60	2.0	3.1 × 1.4	2.2	1.4	round
M. × *dumetorum*	M73	72	2.3	4.9 × 2.7	1.8	1.2	round
M. × *dumetorum*	M74	72	2.5	4.5 × 2.5	1.9	1.4	round
M. × *dumetorum*	M87	60	2.5	3.7 × 1.9	2.0	1.4	round
M. × *dumetorum*	M88	72	2.3	5.0 × 2.6	1.9	1.5	round
M. × *dumetorum*	M90	84	2.8	5.7 × 3.0	2.0	1.5	round
M. longifolia	M31	48	1.5	5.1 × 2.0	2.6	0.6	cordate
M. longifolia	JKM 4875	48	2.1	5.5 × 2.5	2.2	1.0	cordate
M. longifolia	JKM 5219	24	2.1	5.5 × 2.1	2.6	1.1	Sub-cordate
M. aquatica		96	3.6	3.5 × 2.0	1.8	1.0	cuneate
M. aquatica	JKM 4879	96	3.6	3.4 × 2.5	1.4	1.7	cuneate
M. aquatica	JKM 4889	96	3.5	4.9 × 3.6	1.4	2.0	cuneate
M. aquatica	NA 44264	72	3.3	8 × 3.5	1.3	1.3	cuneate
M. × *Piperita*	RAG 13	72	3.4	7.8 × 3.3	2.4	1.2	cuneate

The chromosome numbers of 12 strains of *Mentha* × *dumetorum* studied in the present work are $2n = 60$ and 72 (Table I, Plate 1).

Plate 1 (a) *M.* × *dumetorum* M87, $2n = 60$
(b) *M.* × *dumetorum* M77, $2n = 72$



Studies on meiosis during pollen mother cell formation in *M.* × *dumetorum* indicate irregular meiotic behaviour in 7 of the hybrids examined and complete premeiotic abortion in the remaining 5 strains. All the plants which we examined in which meiosis took place had mainly bivalents and univalents with only a few multivalents. In several cases lagging chromosomes and bridges were observed, and these frequently led to the formation of micronuclei. The formation of unequal tetrads frequently occurred, along with triads, dyads, and occasional monads.

Meiotic chromosome numbers were confirmed for *Mentha aquatica* ($n = 18$) and *M. longifolia* ($n = 12$) with normal meiosis and pollen formation (see Fedorov, An.A., 1969, p366).

All strains of *Mentha* × *dumetorum* examined in this study were sterile and produced no fertile pollen. No seed was set in any of the material grown in the garden which was subject to open pollination.

DISCUSSION

In the hybrid, *Mentha* × *dumetorum,* most morphological characters are found to be intermediate, but values of certain characters were often found to be close to those of one of the parents. Considerable differences in the size of leaves and calyx were observed within the hybrids. It was also noted that hybrid strains in *M.* × *dumetorum* in which there were two or more somatic chromosome numbers (as exemplified by natural material from Leiden, (M26) with $2n = 72$ and M71, synthesized, with $2n = 60$) were morphologi-

cally very difficult to distinguish from one another. These factors make it difficult to predict the origin and parentage of any hybrid on the basis of morphology only, without determining chromosome number. The value of chromosome numbers, as pointed out by Davis and Heywood (1963), is that they often show a high degree of stability.

In *Mentha* × *dumetorum* the chromosomes are small and similar in appearance so that their morphology does not provide a useful character. However, *M.* × *dumetorum* species form a polyploid series so that comparisons of chromosome numbers are useful in determining the parentage of many of the hybrids. In all cases the synthesized hybrids proved to have the chromosome numbers to be expected from their known parentage, these numbers being intermediate between those of the parent species. Thus *M.* × *dumetorum* synthesized by crossing *M. aquatica* ($2n = 96$) with either diploid or tetraploid *M. longifolia* ($2n = 24$ and 48), chromosome numbers of $2n = 60$ and 72 were obtained (Table I). These numbers were also obtained in morphologically similar material of *M.* × *dumetorum* from wild sources.

In *Mentha* × *dumetorum* different chromosome numbers in the parental species involved in the formation of hybrids resulted in irregular meiotic behaviour. This was similar in both the synthesized hybrids and those of wild origin in which meiosis occurred. The occurrence of complete or near complete bivalent formation was observed in all the species, whilst the hybrids showed irregular chromosome asociations — univalents, bivalents, and multivalents — at diakinesis and at metaphase I.

The interspecific hybrids in *Mentha* × *dumetorum* showed 100% sterility, correlated with meiotic irregularities. Snyder (1951) made similar observations on *Elymus* hybrids and associated the cause of sterility with gross meiotic irregularities.

CONCLUSION

A comparison of synthesized hybrids with ones of natural origin on the basis of morphology, chromosome number, meiotic behaviour, and fertility has confirmed the parentage of *Mentha* × *dumetorum* Schultes to be *M. aquatica* × *M. longifolia*.

The following chromosome numbers were obtained for *M. dumetorum* ($2n = 60,72$).

The high pollen sterility recorded for *Mentha* × *dumetorum* in both naturally occurring and synthesized hybrids was correlated with meiotic irregularities. None of the 13 strains of *M.* × *dumetorum* produced any pollen or seed.

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